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CHLAMYDIOLOGY AND RICKETTSIOLOGY

Combined Testing for Chlamydia, Gonorrhea, and Trichomonas by Use of the BD Max CT/GC/TV Assay with Genitourinary Specimen Types

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ABSTRACT The BD Max CT/GC/TV (MAX) assay is a true multiplex assay for simultaneous detection of chlamydia (CT), gonorrhea (GC), and trichomonas (TV). We evaluated assay performance for women using endocervical and vaginal swabs as well as urine specimens. A total of 1,143 women were tested for CT, GC, and TV and, subsequently, another 847 (1,990 total women) for CT and GC only, with positivity rates for CT, GC, and TV of 7.1%, 2.3%, and 13.5%, respectively. In men, the performance for CT and GC was determined using only urine specimens. TV performance was not assessed in male urine samples. Among men, 181/830 (21.8%) and 108/840 (12.9%) chlamydia and gonorrhea infections, respectively, were identified. Comparator assays included BD ProbeTec *Chlamydia trachomatis* Q^x (CTQ)/*Neisseria gonorrhoeae* Q^x (GCQ), Hologic Aptima Combo 2 (AC2) and Aptima TV (ATV), trichomonas microscopy, and culture. MAX CT sensitivity was 99.3% (95% confidence interval [CI], 96.1% to 99.9%), 95.7% (90.8% to 98.0%), 91.5% (85.8% to 95.1%), and 96.1% (92.2% to 98.1%) for vaginal swabs, endocervical swabs, female urine samples, and male urine samples, respectively. MAX GC sensitivity was 95.5% (84.9% to 98.7%), 95.5% (84.9% to 98.7%), 95.7% (85.5% to 99.8%), and 99.1% (94.9% to 99.8%) in the same order. MAX TV sensitivity was 96.1% (91.7% to 98.2%) for vaginal swabs, 93.4% (88.3% to 96.4%) for endocervical swabs, and 92.9% (87.8% to 96.0%) for female urine samples. Specificity for all organisms across all sample types was ≥98.6%. Performance estimates for the MAX assays were consistent with estimates calculated for the comparator assays (all *P* values were >0.1). The availability of a CT/GC/TV multiplexed assay on a benchtop instrument with a broad menu has the potential to facilitate local sexually transmitted infection (STI) testing at smaller laboratories and may encourage expanded screening for these highly prevalent infections.

KEYWORDS *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, molecular diagnostics, sexually transmitted infections

Chlamydia trachomatis and *Neisseria gonorrhoeae* are the two most commonly reported notifiable diseases in the United States (1), and *Trichomonas vaginalis*, while not a notifiable disease, likely causes more sexually transmitted infections (STIs) than chlamydia (CT) and gonorrhea (GC) combined in many populations. The World Health Organization estimates that trichomonas (TV) causes approximately one-half of all curable STIs globally (2, 3). The Centers for Disease Control and Prevention (CDC) recommend chlamydial screening for all women under the age of 25 as well as targeted screening for gonorrhea and trichomoniasis for women at high risk as a result of either behavioral risk or based on subpopulation prevalence (1). While the age distributions

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of chlamydia and trichomonas among women are quite distinct, with chlamydia prevalence peaking among women 15 to 25 and trichomonas prevalence peaking among women 40 to 49, the burden of trichomonas appears to be substantial even among younger women for whom chlamydial screening is recommended (4–8). Continued high infection rates for each of these pathogens, despite ongoing screening programs for women, suggest that efforts to reach men may be important to achieve overall population reductions in disease burden. There are no recommendations for the untargeted screening of men, but screening among high-risk male populations or settings or for men reporting sexual risk factors is recommended for chlamydia and gonorrhea using urine specimens. There are no consensus screening or diagnostic guidelines for trichomonas infection among men.

Infection with *C. trachomatis*, *N. gonorrhoeae*, or *T. vaginalis* is unlikely to cause overt symptoms in the majority of infected women or men, with the possible exception of men infected with *N. gonorrhoeae*. Thus, for populations at risk of disease, screening of asymptomatic populations is critical to overall disease reduction. Further, if undiagnosed, and therefore untreated, each of these STIs has been epidemiologically associated with poor sexual or reproductive health outcomes in women; these include pelvic inflammatory disease (9, 10), tubal factor infertility (11), adverse outcomes during pregnancy (12, 13), or increased risk of HIV acquisition and transmission (14). Screening for asymptomatic infections among at-risk men or women, or diagnosis of infection among symptomatic patients seeking health care, should be performed using nucleic acid amplification tests (NAATs) as recommended by the CDC for chlamydia and gonorrhea (15). Likewise, NAATs also promise to become the gold standard for trichomonas detection, as this is the most sensitive test method available (16–18). Rapid point-of-care options for trichomonas detection among women are available, including wet-preparation microscopy, which performs poorly; a dipstick immunochromatographic assay (OSOM; Sekisui, Lexington, MA); and an isothermal amplification assay (AmpliVue; Quidel, San Diego, CA), of which the latter two perform well (19). None of these tests are useful for the detection of concurrent chlamydia and gonococcal infections and, thus, provide only limited information regarding STI status. Laboratory-based options that can provide all three results are highly desirable for testing women at risk for these STIs. Using the same platform for recommended testing among men is likewise important for lab efficiency by allowing the use of a single diagnostic platform. In this study, we evaluated the performance of the BD Max CT/GC/TV (MAX) assay, a true multiplex test for all three pathogens, using sample types routinely utilized in chlamydia and gonorrhea NAAT assays.

RESULTS

Women. Samples were obtained from 2,166 women; one did not meet eligibility requirements and 51 chose to stop participation prior to collection of all samples, which resulted in a total of 2,114 participants. Prevalence was sufficiently high that sample collection for the trichomonas component of the study was concluded before the chlamydia and gonorrhea components. The trichomonas arm included only 1,291 women because the target number of positive patients had been obtained, and this arm of the study was terminated early. The median age of the 2,144 participants was 26 (range, 16 to 63). Forty-seven percent of women were enrolled from sexually transmitted disease (STD) clinics, 44.5% from family planning clinics, 4.2% from obstetric/gynecologic (OB/GYN) clinics, and 4.4% from other clinical settings (Table 1). Specimens excluded from analyses due to specimen handling or comparator testing protocol deviations at one study site included 278, 281, and 260 vaginal samples, endocervical samples, and urine specimens, respectively. The final sample sizes for each of the analyses are shown in Tables 2 and 3.

In women with evaluable results, chlamydia infections were identified in 141/1,836 (7.7%) vaginal swabs, in 138/1,831 (7.5%) endocervical swabs, and in 142/1,849 (7.7%) urine specimens. The MAX chlamydia sensitivity was 99.3%, 95.7%, and 91.5% for vaginal swabs, endocervical swabs, and urine specimens, respectively. A breakdown by

TABLE 1 Participants and prevalence by study site

Patient sex/clinic	Clinic type	Chlamydia (no. positive/ no. enrolled [%])	Gonorrhea (no. positive/ no. enrolled [%])	Trichomonas (no. positive/ no. enrolled [%])
Women				
Eskenazi Health Services	OB/GYN	12/172 (7.0)	3/172 (1.7)	12/100 (12.0)
Indiana University	STI	13/88 (14.8)	4/88 (4.5)	15/75 (20.0)
Louisiana State University Health Sciences Center	STI	15/165 (9.1)	3/165 (1.8)	22/116 (19.0)
Planned Parenthood, Gulf Coast	Family planning	22/415 (5.3)	4/415 (1.0)	11/74 (14.9)
Planned Parenthood, Southern NE	Family planning	4/160 (2.5)	2/160 (1.3)	3/68 (4.4)
Planned Parenthood, Southeastern Pennsylvania	Family planning	11/283 (3.9)	2/283 (0.7)	25/240 (10.4)
State University of New York, Downstate	Other	4/101 (4)	0/101 (0)	14/68 (20.6)
University of Alabama at Birmingham	STI	61/606 (10.1)	27/606 (4.5%)	52/402 (12.9%)
All participants		142/1990 (7.1%)	45/1990 (2.3%)	154/1143 (13.5%)
Men				
Indiana University	STI	35/106 (33.0)	31/107 (29.0)	Trichomonas testing not done using male urine
Louisiana State University Health Sciences Center	STI	56/278 (20.1)	35/284 (12.3)	
Planned Parenthood, Southern NE	Family planning	7/42 (16.7)	2/42 (4.8)	
State University of New York, Downstate	Other	13/88 (14.8)	6/90 (6.7)	
University of Alabama at Birmingham	STI	70/316 (22.2%)	34/317 (10.7%)	
All participants		181/830 (21.8%)	108/840 (12.9%)	

symptom status is provided in Table 2. The performance estimates did not differ significantly by sample type or presence/absence of symptoms. Specificity was ≥98.6% for all specimen types. The sensitivity estimates for the CTQ and AC2 assays for endocervical swabs were 90.3% and 94.6%, respectively. These were not statistically different from the estimates calculated for the MAX assay ($P = 0.380$) (Table 3). Similarly, the sensitivity estimates for CTQ and AC2 using urine samples were 88.3% and 87.0%, respectively ($P = 0.380$).

Gonococcal infection was identified in women from vaginal swabs, endocervical swabs, and urine specimens in 44/1,836 (2.4%), 44/1,824 (2.4%), and 46/1,849 (2.5%) samples, respectively. MAX sensitivity was 95.5%, 95.5%, and 95.7% in vaginal, endocervical, and urine specimens, respectively. All specificity estimates were ≥99.5%. The performances of the GCQ and AC2 assays were not statistically different from the performance estimated for the MAX assay for GC (Table 3).

Trichomonas was present in 152/1,048 (14.5%) women providing vaginal swabs, 152/1,039 (14.6%) women providing endocervical samples, and 154/1,047 (14.7%) women providing urine specimens (Table 1). Vaginal swabs, endocervical samples, and urine specimens resulted in sensitivity estimates of 96.1%, 93.4%, and 92.9%, respectively (Table 2). The MAX assay detected 3.2% more infections than culture and 71.4% more than wet mount (data not shown). Specificity estimates were ≥97.5%. Lower specificity for the trichomonas assay is likely an artifact of the composite infection standard (CIS) that does not include any amplified comparator assays. The sensitivity and specificity estimated for the MAX trichomonas assay were similar to the estimates for the ATV assay compared to those of the same composite standard based on culture and wet mount ($P = 1.0$) (Table 3). Head-to-head comparisons with each of the reference methods are shown in Table 4. When comparing MAX to the ATV assay, the positive and negative percentage agreements were 93.2% and 99.6%, respectively. The kappa score for overall agreement was 94.7% (95% confidence interval [CI], 92.0% to 97.5%), suggesting excellent agreement between the two molecular assays.

Men. A total of 908 men were enrolled into the study. Sixteen were subsequently found to have not met inclusion/exclusion criteria and were excluded. In addition, due

TABLE 2 BD MAX sensitivity and specificity by sample type compared to infection status for chlamydia, gonorrhea, and trichomonas

	Chlamydia		Gonorrhea		Trichomonas	
Specimen type	Sensitivity[a]	Specificity[a]	Sensitivity[a]	Specificity[a]	Sensitivity[a]	Specificity[a]
Vaginal swab						
Asymptomatic	100 [51/51] (93.0–100)	98.7 [734/744] (97.5–99.3)	94.1 [16/17] (73.0–99.0)	99.9 [777/778] (99.3–100)	93.1 [27/29] (78.0–98.1)	97.5 [270/277] (94.9–98.9)
Symptomatic	98.9 [89/90] (94.0–99.8)	98.6 [938/951] (97.7–99.2)	96.3 [26/27] (81.7–99.3)	99.8 [1,012/1,014] (99.3–99.9)	96.7 [119/123] (91.9–98.2)	99.5 [616/619] (98.6–99.8)
Total	99.3 [140/141] (96.1–99.9)	98.6 [1,672/1,695](98.0–99.1)	95.5 [42/44] (84.9–98.7)	99.8 [1,789/1,792] (99.5–99.9)	96.1 [146/152] (91.7–98.2)	98.9 [886/896] (98.0–99.4)
Endocervical swab						
Asymptomatic	94.1 [48/51] (84.1–98.0)	99.1 [737/744] (98.1–99.5)	94.1 [16/17] (73.0–99.0)	100 [777/777] (99.5–100)	96.6 [28/29] (82.8–99.4)	98.2 [270/275] (95.8–99.2)
Symptomatic	96.6 [84/87] (90.3–98.8)	99.4 [943/949] (98.6–99.7)	96.3 [26/27] (81.7–99.3)	99.9 [1,002/1,003] (99.4–100)	92.7 [114/123] (86.7–96.1)	99.8 [611/612] (99.1–100)
Total	95.7 [132/138] (90.8–98.0)	99.2 [1,680/1,693] (98.7–99.6)	95.5 [42/44] (84.9–98.7)	99.9 [1,779/1,780] (99.7–100)	93.4 [142/152] (88.3–96.4)	99.3 [881/887] (98.5–99.7)
Female urine						
Asymptomatic	92.3 [48/52] (81.8–97.0)	99.7 [747/749] (99.0–99.9)	88.9 [16/18] (67.2–96.9)	99.5 [779/783] (98.7–99.8)	93.1 [27/29] (78.0–98.1)	98.2 [272/277] (95.8–99.2)
Symptomatic	91.1 [82/90] (83.4–95.4)	99.4 [952/958] (98.6–99.7)	100 [28/28] (87.9–100)	99.9 [1,019/1,020] (99.4–100)	92.8 [116/125] (86.9–96.2)	99.8 [615/616] (99.1–100)
Total	91.5 [130/142] (85.8–95.1)	99.5 [1,699/1,707] (99.1–99.8)	95.7 [44/46] (85.5–98.8)	99.7 [1,798/1,803] (99.4–99.9)	92.9 [143/154] (87.7–96.0)	99.3 [887/893] (98.5–99.7)
Male urine						
Asymptomatic	98.6 [69/70] (92.3–99.7)	99.5 [378/380] (98.1–99.9)	80.0 [4/5] (37.6–96.4)	100 [447/447] (99.1–100)	No evaluation of trichomonas testing was performed in male urine	No evaluation of trichomonas testing was performed in male urine
Symptomatic	94.6 [105/111] (88.7–97.5)	99.3 [267/269] (97.399.8)	100 [103/103] (96.4–100)	100 [285/285] (98.7–100)		
Total	96.1 [174/181] (92.2–98.1)	99.4 [645/649] (98.4–99.8)	99.1 [107/108] (94.9–99.8)	100 [732/732] (99.5–100)		

[a]Values shown are percentage [number of positive results/number of infections] (95% confidence interval).

TABLE 3 Performance of female samples when comparing the BD MAX to CTQ/GCQ and Aptima assays (AC2 and ATV) using a rolling infection status calculation

Disease/sample type	Sensitivity				Specificity			
	MAX[a]	Q[x] assays[a]	Aptima assays[a]	*P* value	MAX[a]	Q[x] assays[a]	Aptima assays[a]	*P* value
Chlamydia								
Endocervical	95.7 [132/138] (90.8–98.0)	90.3 [131/145] (84.4–94.2)	94.6 [135/145] (87.8–96.2)	0.414	99.2 [1,681/1,694] (98.7–99.6)	99.6 [1,701/1,708] (99.2–99.8)	99.4 [1,701/1,711] (98.9–99.7)	0.380
Female urine	91.5 [130/142] (85.8–95.1)	88.3 [128/145] (82.0–92.5)	87.0 [127/146] (80.6–91.5)	0.414	99.5 [1,699/1,707] (99.1–99.8)	99.8 [1,709/1,713] (99.4–99.9)	99.6 [1,701/1,708] (99.2–99.8)	0.380
Gonorrhea								
Endocervical	95.5 [42/44] (84.9–98.7)	97.8 [45/46] (88.7–99.6)	93.6 [44/47] (82.8–97.8)	0.760	99.9 [1,780/1,781] (99.7–100)	99.7 [1,801/1,807] (99.3–99.8)	99.9 [1,808/1,810] (99.6–100)	0.170
Female urine	95.7 [44/46] (85.5–98.8)	97.8 [45/46] (88.7–99.6)	93.6 [44/47] (82.8–97.8)	0.760	99.7 [1,798/1,803] (99.4–99.9)	99.7 [1,806/1,812] (99.3–99.8)	99.9 [1,805/1,807] (99.6–100)	0.170
Trichomonas								
Vaginal swabs	96.6 [143/148] (92.3–98.5)	Not done	98.0 [145/148] (94.2–99.3)	1.0	98.8 [858/868] (97.9–99.4)	Not done	98.2 [852/868] (97.0–98.9)	1.0

[a]Values shown are percentage [number of positive results/number of infections] (95% confidence interval).

TABLE 4 Head-to-head comparison of MAX TV and other assays for detection of trichomonas

Test type	MAX TV (−)	MAX TV (+)	Percent agreement[a] (95% CI)	Overall agreement (95% CI)
Wet prep (−)	889	68	NPA = 92.9 (91.9–94.6)	93.2 (91.5–94.6)
Wet prep (+)	3	88	PPA = 96.7 (90.8–98.9)	
Culture (−)	886	11	NPA = 98.8 (97.8–99.3)	98.4 (97.4–99.0)
Culture (+)	6	145	PPA = 96.0 (91.6–98.2)	
ATV (−)	852	3	NPA = 99.6 (99.0–99.9)	98.6 (97.7–99.2)
ATV (+)	11	150	PPA = 93.2 (88.2–96.1)	

[a]Positive (PPA) and negative (NPA) percent agreement calculated on the presumption that the reference method is 100% accurate.

to noncompliance with specimen collection or unavailable CT/GC comparator results, 62 and 52 men did not have specimens tested, respectively. The final sample size for analysis was 830 and 840 for chlamydia and gonorrhea, respectively. Chlamydial infections were identified in 181 (21.8%) participants while gonococcal infections were detected in 108 (12.9%) men (Table 1). The MAX CT/GC assay detected 174/181 (96.1% [95% CI, 92.2% to 98.1%]) chlamydial infections and 107/108 (99.1% [95% CI, 94.9% to 99.8%]) gonococcal infections. Specificity was greater than 99% for both organisms (Table 2).

Mixed infections. Finally, as with any true multiplex assay, it is important to assess the impact of mixed infections on the assay's ability to detect all of the pathogens present. In this study, 34/1,849 (1.8%) women had coinfections with two or more organisms while 35/830 (4.2%) men had both chlamydia and gonorrhea. For women with chlamydia, vaginal swab sensitivity in the absence of coinfection was 100% (111/111), 94.4% (17/18) in the presence of gonorrhea, and 100% (16/16) in the presence of trichomonas. For gonorrhea, vaginal swab sensitivity was 95.8% (23/24) for women without coinfection and 94.4% (17/18) and 100% (6/6) for women with chlamydial and trichomonal coinfections, respectively. For women infected with trichomonas, vaginal swab sensitivity was 95.5% (128/134) for those without coinfection and 100% for those with concomitant chlamydia (16/16) or gonorrhea (6/6). Among men, for those with chlamydial infections, the sensitivity of urine was 98.0% (144/147) for a single infection and 88.2% (30/34) when gonorrhea was also present. For men with gonococcal infections, sensitivity estimates were 100% and 97.1% for men with only gonorrhea (73/73) versus those who also had chlamydia (34/35). Performance estimates were not significantly different in the presence or absence of coinfection in the study population (data not shown).

DISCUSSION

In this study, MAX performance was equivalent to the performances of currently available platforms used in many centralized reference laboratories (Table 3). Sensitivity and specificity estimates for each of the reference assays were estimated using the other two assays' results as well as the results from MAX. This rolling patient infection status (PIS) comparison, such that an assay being evaluated never contributes to the PIS, has been commonly used in evaluations of STI NAATs (20–22). The sensitivity and specificity of these assays for vaginal swabs, endocervical swabs, and first-catch urine samples were comparable to performances when testing these same specimens on Becton Dickinson BD Qx assays on the Viper System and Hologic AC2 and ATV assays on the Tigris/DTS systems. As has been shown for other assays, for diagnosis of chlamydial infections, the MAX assay using self-obtained vaginal swab specimens provided the most sensitive means of detection of chlamydial infections, followed by endocervical swabs. Self collection of vaginal swabs is recommended by the CDC for reasons of convenience as well as performance, and many newer diagnostic assays (20, 21, 23) have evaluated only this sample type and not clinician-collected vaginal swabs similar to the study reported here. Urine samples, while not significantly less sensitive than the other specimen types, detected fewer positives overall than the two types of

swab specimens. Lower sensitivity when using urine specimens was seen predominately for the detection of gonorrhea with specimens from asymptomatic patients who may be expected to have lower organism loads. Further, among both men and women, the number of asymptomatic gonococcal infections was quite small as discussed above. However, despite low estimates among this subset of specimens, the sensitivity and specificity estimates for urine samples compared well with those of the CTQ/GCQ and AC2 assays (Table 3).

While the estimate for gonococcal sensitivity among asymptomatic men was low (80.0%), only 5/142 (3.5%) asymptomatic infections were detected, compromising the reliability of the estimate. Based on our results, however, the prevalence of asymptomatic infections was so low that increasing the sample size in order to tighten the confidence interval was not practical. For chlamydia, this was not the case since the overall number of infections was higher and, in that case, the confidence intervals suggest excellent performance. The most likely explanation for variance in gonorrhea positivity is that the organism load is low in urine samples from asymptomatic men as is likely for women. It is very promising that even among this group of men with only a 1% positivity rate, the specificity is sufficiently high that the MAX assay can be expected to have an excellent positive predictive value.

In this study, as in other evaluations of trichomonas culture sensitivity (18, 24), there was a slight benefit to performing NAATs (Table 4); however, this is based on 5 days of reading the InPouch and is, thus, slow, labor-intensive, and requires highly skilled microscopists. Further, the lack of a standard that includes NAATs is known to inflate the sensitivity estimates of culture, and thus the marginal difference in sensitivity would likely expand to a larger difference if a composite infection standard (CIS) based on multiple NAATs had been possible during this trial. While the performance of wet-prep microscopy was extremely poor, there is still a utility to performing on-site, rapid evaluations that can at least identify some women needing treatment. Some clinics that use NAATs for TV also use wet prep as a triage method, whereby swabs from positive women are sent only for chlamydia/gonorrhea testing while wet-mount negative samples are tested by NAATs for all three pathogens.

A limitation of this trial was that men were not tested for TV; however, at this time there are no widely accepted recommendations for TV screening in men. Diagnosis and management of men with trichomoniasis is an important topic for future clinical and translational research. Recently, the GeneXpert TV assay (Cepheid, Sunnyvale, CA) was approved for detection of trichomonas in male urine samples. As more molecular diagnostics are cleared for use with male urine samples, more data regarding the prevalence of infection in different populations will become available to inform national policy regarding this pathogen.

In summary, we found that for diagnosis of three of the most common STIs in women, chlamydia, gonorrhea, and trichomoniasis, the MAX platform provided high sensitivity and specificity using vaginal or endocervical swabs or urine specimens. In many U.S. settings, given the broad utility of the platform based on current and future menus, the MAX offers a potential solution for small to medium laboratories, which may keep testing local and potentially increase routine screening for common STIs.

MATERIALS AND METHODS

Women were recruited from eight STI, family planning, and OB/GYN clinics located throughout the United States (Table 1). Five of these sites also recruited men. For men and women, eligibility criteria included presenting for routine STI symptom evaluation or screening and being of appropriate age to provide informed consent for research. Exclusion criteria included the use of antibiotics, including metronidazole/tinidazole within the previous 14 days, having urinated within 1 h prior to recruitment, and additionally for women hysterectomy or use of contraceptive foams or jellies within 8 h of recruitment. The protocol was reviewed and approved by institutional review boards at each participating institution, and informed consent was obtained prior to sample collection. Women were asked to provide, in the order described, a first-catch urine sample, a self-obtained vaginal swab (for use with the MAX assay), 3 clinician-collected vaginal swabs, and 3 endocervical swabs (Fig. 1A). Men provided a urethral swab and a urine specimen (Fig. 1B). Urine specimens were aliquoted into MAX and comparator assay urine sample transport devices according to package insert recommendations. Urine specimens for men and women were tested using MAX, the BD ProbeTec *Chlamydia trachomatis* Q^{x} (CTQ) (20) and

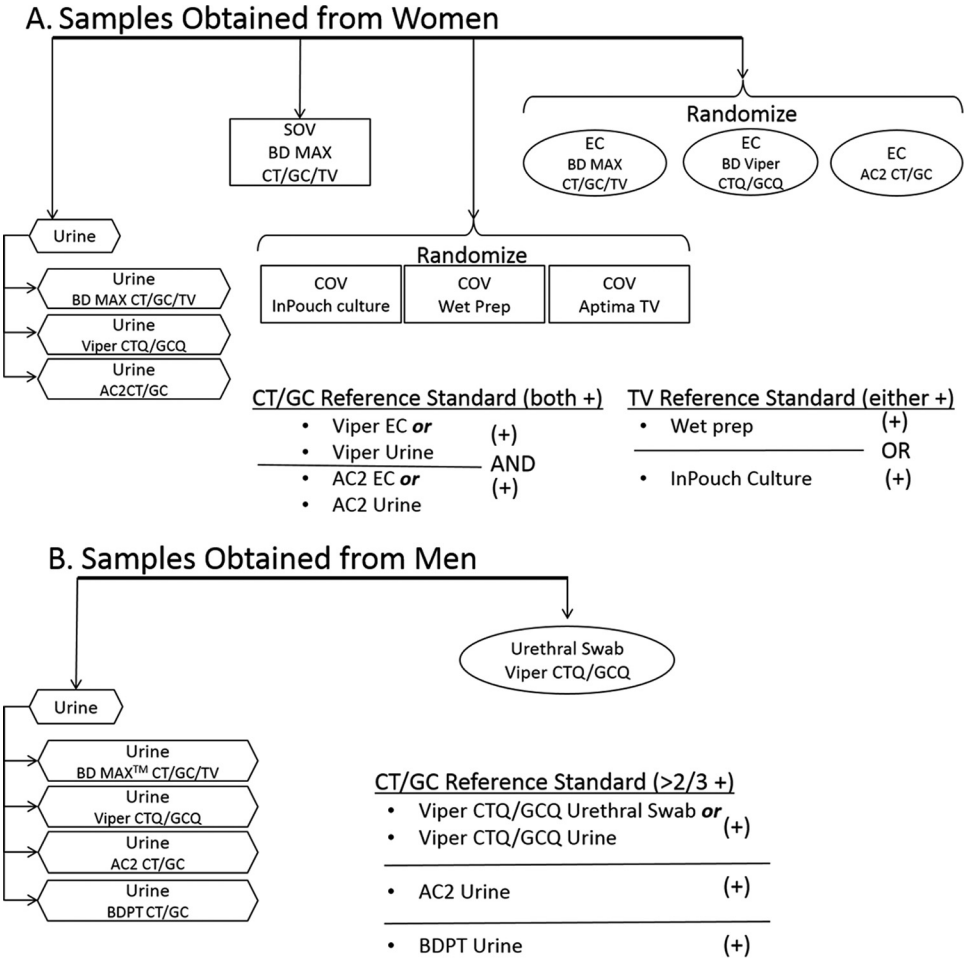

FIG 1 Sample collection scheme and definition of patient or composite infection status. (A) Samples from women where self-obtained vaginal swab (SOV), clinician-obtained vaginal swab (COV); and endocervical swab (EC). (B) Samples from men.

Neisseria gonorrhoeae Q^{x} (GCQ) assays (23) performed on the BD Viper (BD Diagnostics, Sparks, MD), and the Aptima Combo 2 (AC2) chlamydia/gonorrhea assay (25) on the Tigris/DTS system (Hologic, San Diego, CA). For men, an additional urine test was performed using the BD ProbeTec CT/GC (BDPT) assay (26). The first and second clinician-obtained vaginal swabs were used in randomized order for wet-prep microscopy or InPouch TV culture (Biomed Diagnostics, Santa Clara, CA) (27) while the third was used for the Aptima TV (ATV) assay (Hologic, San Diego, CA) (18). The three endocervical swabs were randomized for use with MAX, CTQ/GCQ, and AC2. Male urethral swabs were tested using the CTQ/GCQ assay. Specimens were excluded from analyses as a result of improper sample collection or storage, incomplete sample collection, noncompliant testing, or when no results were available for that sample.

The MAX assay is a TaqMan-based PCR assay that utilizes target-specific primers and probes to perform simultaneous amplification and detection of amplified products using quenchers and fluorophores. The test utilizes processing strips, each of which contains a place for insertion of the sample tube followed by a series of tubes, including an extraction tube containing dried magnetic affinity beads, protease reagents, and a sample processing control; a tube with dried master mix; a tube with dried primers and probes; three tubes with required rehydration buffers; and a tube to hold waste as well as the pipette tips necessary to perform all of the liquid handling processes. Each strip is utilized for a single patient sample and provides chlamydia, gonorrhea, and trichomonas results simultaneously. Technician hands-on time, approximately 15 min, is limited to specimen processing and insertion of the specimen tube in the processing strip. Two racks that hold up to 12 strips each can be run on the instrument, and a disposable cartridge with channels in which the amplification and detection occurs can run up to 24 tests in a run. These cartridges can be used once with 24 samples or on two separate runs processing up to 12 samples on each run. The total time from for processing, amplification, and detection, with results released at the end of the run, is approximately 3 h.

For this study, women were defined as symptomatic for chlamydia/gonorrhea analyses if they reported dysuria, abnormal discharge, pelvic pain, or coital discomfort. All women not reporting these symptoms were classified as asymptomatic for analyses. Symptoms of trichomonas were defined differently and included abnormal discharge, dysuria, itching, or odor. Women not reporting these

symptoms were classified as asymptomatic for trichomonas analyses. Therefore, the numbers of symptomatic and asymptomatic women differ in the analytic data sets for performance estimation of the chlamydia, gonorrhea, and trichomonas assays. Men were categorized as symptomatic if they complained of discharge, burning on urination, or testicular pain. All other men were considered asymptomatic.

For evaluation of chlamydia and gonorrhea test performance in women, infections were defined using a patient infection status (PIS) that attempts to identify infection inclusive of both possible genitourinary sites of infection (i.e., cervical versus urethral infections). The PIS defined chlamydial or gonococcal infections based on the positive results of two comparator assays (CTQ/GCQ and AC2) using results from both endocervical swabs and urine specimens. At least one positive result, from either sample type, was required from each assay in order to categorize a participant as infected. For trichomonas, vaginal infections were defined by a composite infection standard (CIS), used when evaluating only a single sample type. CIS-defined infections were comprised of either positive wet-preparation microscopy results or a positive culture. The ATV assay was not used to define infections, but a head-to-head comparison was performed. The rationale for this decision was that the ATV result would have had to occur in conjunction with either a wet prep or a culture-positive result in order to define infection. Thus, using only wet prep and culture and allowing a single positive by either assay to define infection provides exactly the same infection status categorization as would have been obtained if ATV had been included in the definition. A head-to-head comparison of MAX TV and ATV results is provided separately since both of these assays are assumed to be more sensitive than either wet prep or culture. For men, a CIS was used since urethral swabs and urine capture infection at the same body site. Infections were defined by positive results from $\geq$2 of the 3 assays performed on the 4 specimens (for 2 specimens [1 urethral swab and 1 urine specimen], the CTQ/GCQ assay was performed) (Fig. 1B). Thus, both CTQ assay results alone did not define an infection, as at least one other assay-positive result was required.

The score method was used to calculate 95% confidence intervals (CIs) for sensitivity and specificity estimates. Logistic regression using generalized estimating equations, to control for repeated measures, was performed to compare the performance characteristics of the comparator assays in this patient population, with the estimates obtained for the MAX assays with an alpha of 0.05. Estimates for the performances of the CTQ/GCQ assays were based on PIS derived using AC2 and MAX as comparators, while the AC2 was compared to a PIS comprised of CTQ/GCQ and MAX results as comparators. These comparisons can only be performed for endocervical and urine specimens since vaginal swab results were not available for the CTQ/GCQ and AC2 platforms for CT/GC. For TV, the performance estimates were calculated for both MAX and ATV using the same composite infection standard of culture and/or wet prep. Kappa scores were calculated using Cohen's method for a head-to-head comparison of agreement for the MAX TV and ATV assays.

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